Ratios Matter

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Ecological Stoichiometry of Elements Beyond C:N:P

In this issue of *Ratios Matter*, we are excited to widen our scope and give you a look into the less researched side of ecological stoichiometry – the side that includes other elements! The established field of ecological stoichiometry has largely revolved around the three important elements: C, N, and P. Recent studies, however, have highlighted the importance of considering other elements beyond C, N and P such as calcium, cobalt, arsenic, zinc, and many more. We highlight work on several of these lesser-studied elements in this issue of *Ratios Matter*. It is great to see that stoichiometrists working on other elements are no longer in a galaxy far, far away, but walking and researching among us!

Ratios Matter is pleased to have this guest commentary contributed by **Puni Jeyasingh** of Oklahoma State University. For information on Puni and his research, visit his website.

Stoichiometry of Other Elements in Response to Nutrient-Limited Growth

External supplies and internal concentrations of phosphorus interact to affect growth (Droop 1973). While the growth rate hypothesis (GRH; Elser et al. 1996) illuminates some of the biochemical mechanisms underlying Droop dynamics, genomic methods capturing the entire physiological status of organisms grown under contrasting P supplies show widespread effects across the metabolic chart (see Jeyasingh et al. 2011 and references therein). This indicates that the processing of multiple other elements should also vary. Indeed, agronomists interested in maximizing growth of crop varieties have found that models with P supply and P content perform poorly in predicting growth compared those using data on the entire suite of biogenic elements in a cell or individual (i.e., its ionome; Salt et al. 2008). Studies in the field of ionomics have proliferated in the last decade and led Baxter (2015) to posit that elements of the ionome do not behave independently and that such combinations of elements should be considered for a better understanding of growth. *Continued on page 8*.

Remember the Frontiers Research Topic on Ecological Stoichiometry?

To date, this significant compilation has

Check it out for yourself at

www.frontiersin.org/research-topics/4961

Save the Date

Unifying Ecology Across Scales

Gordon Research Conference *July 22-27, 2018*

Ecology is the science of living systems with a vast empirical domain that includes a single protist searching for resources to the sustainability of the global ecosystem. As one species (i.e., humans) accelerates change in the planet's ecology, scientists still struggle to understand the laws governing living systems that span such broad scales of space, time and biological organization.

This GRC conference builds on recent efforts to unify understanding of disparate ecological systems and clades across scales into a single framework. Despite major advances based on studying flows of energy and materials across scales from organism to ecosystem, leading unifying theories spanning scales of ecological organization have yet to formally integrate existing knowledge about one of the very things that makes living systems "living": information.

This conference is open to everyone. Apply before **June 24, 2018** (but earlier is better).

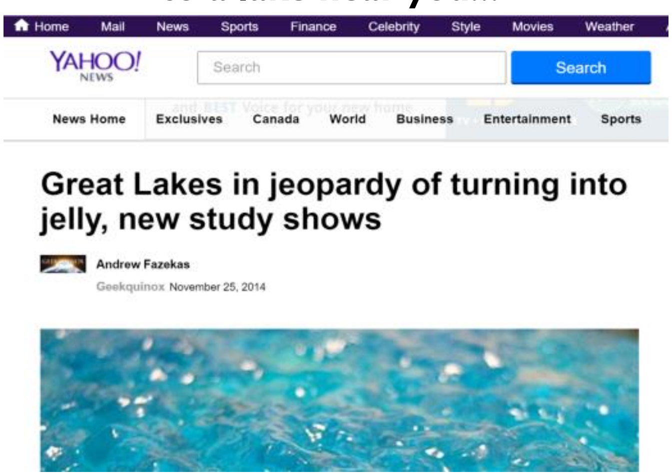
For more information: *www.grc.org/unifying-ecology-across-scales-conference/2018/*

The Story of Declining Calcium

There have been striking declines in concentrations of aqueous calcium (Ca) in many soft-water lakes over the past 30 years. These declines appear linked to forest recovery from past acid deposition, which depleted Ca in soils. Less acid deposition reduced loss rates of soil Ca from catchments and lower export to downstream lakes. Over the next 20-30 years, lake Ca concentrations are expected to continue to decline with possible cascading effects on aquatic ecosystems due to the loss of vulnerable taxa.

Calcium is a critical component of structural materials such as bones, carapaces, scales and shells and is especially essential for invertebrates with calcified exoskeletons. For zooplankton, Ca can be acquired by direct uptake from the water but may also be derived from prey consumption. Low Ca concentrations, in the suboptimal range, compromise remineralisation of the exoskeleton and can constrain reproduction and survival. These effects of low Ca may be most profound during post-molt, which is a period of greatest Ca uptake. Hence, relatively low Ca concentrations may sustain a mature individual but may not be adequate for juvenile survival, which can create developmental "calcium bottlenecks" in populations.

The world of jelly may be coming to a lake near you...



Susceptibility to Ca-limitation may vary among taxa within zooplankton communities. Different species may have different Ca demands, which would make some particularly vulnerable to low Ca. For example, some *Daphnia* clones have especially Ca-rich exoskeletons, which must be regenerated after each molt. Such species could be at a competitive disadvantage relative to less Ca-rich competitors, *Holopedium* or *Bosmina*. Recent increases in *Holopedium* (called "jellification" by some) of north temperate lakes in Canada may be due to decreased Ca concentration.

What about stoichiometry? Most previous work examining the effects of Ca deficiency on zooplankton has focused on Ca alone. However, in a recent article, Prater *et al.* (2016) found that effects of low Ca concentrations on *Daphnia* varied with food P content. While daphnid growth was linked to dietary P, Ca limitation was more strongly linked with survival. This study also found body Ca content may not be a good indicator of Ca demand because animal body Ca content varied strongly with growth rate. Multi-elemental nutritional requirements of zooplankton should receive future consideration and may alter our evolving view of Ca and its importance in food webs.

Contributed by Cecilia Laspoumaderes

Prater et al. (2016). Effects of calcium and phosphorus limitation on the nutritional ecophysiology of *Daphnia*. Limnol. Oceanogr. 61: 268-278

Stoichiometry of Trace Elements in Plants

Your parents likely encouraged you to eat certain vegetables for the minerals that they contain, but did you ever wonder why some plants are rich in iron and others are rich in zinc? There is a wealth of knowledge about influences on the C, N, and P content of plants, but micronutrients and trace elements have received much less emphasis. This issue of *Ratios Matter* highlights the importance of ~22 'other' elements that are required by plants and animals. Interest in these elements is expanding rapidly due to advances in analytical tools for measuring tissue nutrient content (Salt et al. 2008) and increased recognition of the importance of co-limitation in ecology.

A recent paper by Ana Campos et al. (2017) examined how the availability of zinc (Zn) affected the ionome of the plant, *Arabidopsis*. It should come as no surprise that Zn limitation caused a decrease in the concentration of Zn in *Arabidopsis*. However, limitation by Zn also caused *Arabidopsis* to dramatically increase its tissue nutrient content of several elements (Mg, Fe, Cd, B, P, Ca, and Mn) and decrease its content of others (Cu and Na). There are several other studies that yield qualitatively similar results, yet the underlying biological mechanisms remain unidentified. A promising inroad to understanding these physiological responses is a 'multi-omic' approach that couples ionomic analyses with metabolomic or transcriptomic methods. Zhaotang Ding et al. (2017) subjected tea plants to P-limitation and found that changes in the tissue concentrations of five elements (S, Mn, Fe, Zn, and Cu) were related to changes in the concentration of key metabolites.

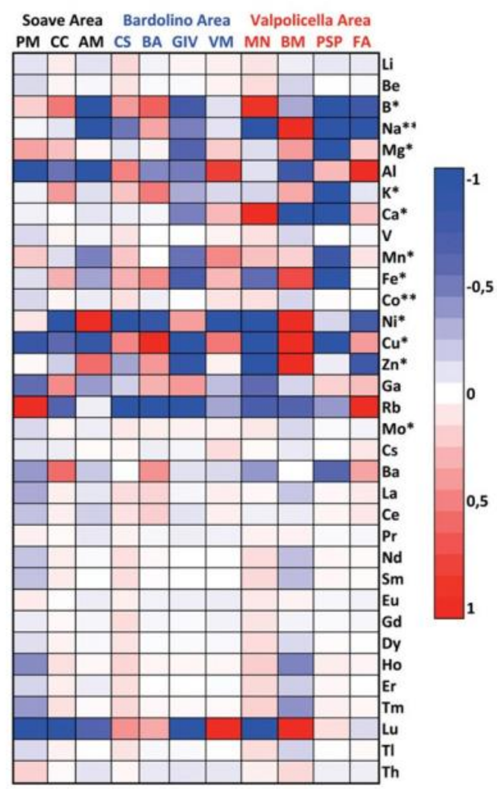


Figure 1 from Pii et al. (2017) showing a heatmap of elemental concentrations in wine grapes from different areas and vineyards.

Plant ionomes can be used to verify the provenance of agricultural products. Youry Pii et al. (2017) recently reviewed how concentrations of trace elements could be used to determine the regional and sub-regional origins of wine. There, now you have another reason to bring up ionomics at scientific conference mixers!

Contributed by Casey Godwin

Campos, A., W. Kruijer, R. Alexander, R.C. Akkers, J. Danku, D.E. Salt & M.G.M. Aarts. 2017. Natural variation in Arabidopsis thaliana reveals shoot ionome, biomass, and gene expression changes as biomarkers for zinc deficiency tolerance. J Exp Bot 68:3643-3656.

Ding, Z., S. Jia, Y. Wang, J. Xiao & Y. Zhang. 2017. Phosphate stresses affect ionome and metabolome in tea plants. Plant Physiol Biochem 120:30-39.

Pii, Y., A. Zamboni, S. Dal Santo, M. Pezzotti, Z. Varanini & T. Pandolfini. 2017. Prospect on ionomic signatures for the classification of grapevine berries according to their geographical origin. Front Plant Sci 8:640.

Salt, D.E., I. Baxter & B. Lahner. 2008. Ionomics and the study of the plant ionome. Annu Rev Plant Biol 59:709-733.

The Stoichiometry of Cobalt in the Ocean

There remains a lot to learn about the cycling of cobalt (Co) in the oceans. Using the GEOTRACES database that includes sampling stations across the Northern and Southern Atlantic Ocean, Saito et al. (2017) investigated the stoichiometry of cobalt (Co) in relation to the macronutrient P across the Atlantic Ocean. They found a positive relationship between Co and P irrespective of where the water was collected. Integrating water samples throughout the vertical profile revealed Co:P ratios that span between approximately 20 to 70 µmol:mol^{-1}. However, these integrated samples do not reveal processes that occur at different depths. In the top 300 m, especially in very low P areas of the ocean, the dissolved and particulate Co:P ratios are orders of magnitude higher and approach Co:P 500 µmol:mol^{-1}. This study stresses the importance of examining more than macro nutrient requirements by revealing a much higher cellular demand for Co in P limiting waters. The increased demand for Co in very low P environments may be because Co can be used to make alkaline phosphatase, a phosphate scavenging enzyme. This work shows the importance of studying how these two very different elemental pools interact.

From the paper: "With the smallest inventory of any required nutritional element in the oceans and its potential for biochemical substitution, dissolved Co stoichiometries found in the oceans appear to be among the most dynamic of any element used by life"

Contributed by Nicole Wagner

Saito, M.A., A. Noble, N. Hawco, B.S. Twining, D.C. Ohnemus, S.G. John, P. Lam, Conway, R. Johnson, D. Moran and M. McIlvin. 2017. The acceleration of dissolved cobalt's ecological stoichiometry due to biological uptake, remineralization, and scavenging in the Atlantic Ocean, Biogeosciences: doi:10.5194/bg-2016-511

JOIN THE DARK SIDE OF STOICHIOMETRY

Stoich-Comic
by Judith Sitters

Profiles in Stoichiometry
9 Questions for Keeley MacNeill

Tell us about your scientific background and how you became interested in ecology. I was preparing for a medical career when I took John Schade's Ecology class at St. Olaf College (Minnesota, USA). This led to two summers of research with John at the Angelo Coast Range Preserve near Branscomb, California. Inspired by the prospect of making a career out of asking questions, I spent several years after college but before graduate school gaining research experience with work on eco-evolutionary feedbacks in Trinidad and stoichiometry as a driver of respiratory quotient variation in Norway. I'm currently at Cornell University (New York) where I'm working on my PhD with Alex Flecker.

Do you remember when you first heard about ecological stoichiometry? During my first summer at the Angelo, I remember lying in the hammock at the field station and reading *Ecological Stoichiometry* for our picnic table literature discussions. The project I worked on the next summer used ES as a framework to examine N:P as a driver of nutrient uptake in stream ecosystems. I have always been fascinated by ES as a framework, and not only does my research keep returning to it, but during seminars and informal discussions with other researchers, I'm constantly asking, "Have you thought about the stoichiometry?" It has resulted in some interesting conversations and even a collaboration on pollen stoichiometry.

What is your current research on stoichiometry? The most recent part of my PhD research focuses on N:P as a driver of arsenic (As) retention vs. transport in streams. We've long known that because AsO_4^{3-} and PO_4^{3-} have similar shapes, AsO_4^{3-} can be taken into cells in place of PO_4^{3-} and that more AsO_4^{3-} is taken up when P supplies are low. My research shows that AsO_4^{3-} uptake depends on dissolved N:P ratios and that more AsO_4^{3-} is taken up in high N:P environments. Currently, I'm researching how N:P availability affects transfer and retention of As in stream food webs with effects on detoxification and downstream transport.

Do you view ecological stoichiometry as its own research field? Why or why not? I'm not sure that I consider anything to truly stand alone as it's own field, particularly given the push and need for integrative research. However, as far as any field stands alone, I think ES is absolutely it's own field. There's still an infinite amount of valuable research to be done, both within ES as it's own field and to contribute to other fields.

What is it like to work on an element beyond C:N:P? Exciting! This is particularly true given the toxic nature of As and the implications for human health given my original desire to be a medical doctor. I've always been fascinated by ES but this has added another layer of complexity and intrigue, and it has opened up a lot of doors to exciting and new research projects.

What is your favorite element? Arsenic! I would have said P until I found out that As can trick cells into thinking it's P.

9 Questions Continued

What is your favorite stoichiometry paper and why? A favorite is tough, but I was recently excited by Kaspari & Powers (2016)*. They presented a well-framed 'state of stoichiometry' paper, grounded in the history of stoichiometry and showing where the field is headed. They focused on essential elements and expanding the framework out from CNP. This resonated with me as much of my research focuses on As, a lesser studied toxic element with important implications for the cycling of more common elements.

*Kaspari, M. & J.S. Powers. 2016. Biogeochemistry and geographical ecology: Embracing all twenty-five elements required to build organisms. Am Nat. 188, Suppl 1:S62-73

Where do you see ecological stoichiometry progressing in the next 10 years, and do you see elements beyond CNP becoming more of a focus? I'm biased, but I agree with Kaspari & Powers, and other people who have shown the importance of including lesser-studied elements in ES (Mb+N, Se+Hg, etc). While I think stoichiometry makes the world go-round, focusing on just a few elements isn't enough to fully explain many of the processes we examine. I also think integrating the biogeochemical side of ecological stoichiometry with the nutritional ecology side is an important goal to continue working towards. For me, thinking about the physiology of organisms and the role P plays has been important for researching the effects of As when it replaces P.

If arsenic were an animal, what would it be? If As was an animal, it would be a coral snake and P would, of course, be a milk snake. If an animal eats a milk snake (P), it has a yummy, nourishing meal. However, if it accidentally gets a coral snake (As) because it looks the same, it's in for an unpleasant surprise! Also, the relative abundance of the snakes determines the likelihood of a consumer being poisoned; although more research is clearly needed on the stoichiometry of milk and coral snakes and their effects on consumers.

Stoichiometric Applications from the World of Food Science

Do you know the origin of your food? With premium prices for organic and wild-caught game, fraudulent food labelling practices are commonplace. Believe it or not, stoichiometry could prove useful in determining the origin of your food (and apparently your wine too, see pg. 4). In a new study by Danezis et al. (2017), researchers used highly sensitive inductively coupled plasma mass spectrometry to generate extensive elemental profiles (~39 elements). These profiles were used to distinguish among wild-caught, farm raised, and domestically raised rabbits. In general, rare earth elements (e.g., Ho, Tm, and Lu) were more abundant in wild-caught animals and these elements were much better at discriminating between rabbit groups than trace elements (e.g., Sr, V, and Co). This study shows the potential power of using novel multi-elemental approaches in an applied setting. **Coming to a supermarket near you...**

Danezis, G.P., A.C. Pappas, E. Zoidis, G. Papadomichelakis, I. Hadjigeorgiou, P. Zhang, V. Brusic and C.A. Georgiou. 2017. Game meat authentication through rare earth elements fingerprinting. Analytica Chimica Acta

Continued from page 1.

Although, as this issue of *Ratios Matter* highlights, the growth-relevance of elements other than nitrogen and phosphorus are becoming increasingly appreciated, we have yet to systematically characterize the responses of other elements as growth varies as a function of external P supply and internal P quota. Short of such systematic data, are there general trends in the ionomes of P-limited and P-replete organisms? My initial screening of such studies (www.ionomicshub.org; and datasets generated in our lab) indicated that: (i) P-limitation increases carbon quota, and (ii) P-limitation decreases the quota of all other elements (Jeyasingh, in prep). The primary physiological mechanisms for higher C under P-limitation in autotrophs has been identified (e.g., Brembu et al. 2017), as are its consequences for consumer growth and higher order ecology (e.g., Hessen & Anderson 2008). Comparatively little is known about the physiological basis for lower concentrations of other elements, notably trace metals. One hypothesis is that lower rates of protein synthesis under P-limitation would render them replete. But too much trace metals can quickly become toxic and chelating them or pumping them outside the cell is energetically expensive (Crichton 2017). Thus, the growth penalty observed under P-limitation might not only be due to P-limitation of ribosome biogenesis, or the handling of excess carbon, but also due to handling of multiple other elements that can alter energetic budgets substantially. A more inclusive understanding of the costs involved in such responses, and how they vary among taxa with differential elemental demands and growth maxima or ecosystems with differential elemental supplies and productivity, would enrich our ability to understand ecological dynamics using the framework of ecological stoichiometry.

One reason for the lack of focus on other elements is logistical challenges in reliably measuring them. Advanced analytical technology (e.g., inductively coupled plasma spectroscopy) is becoming increasingly reliable and affordable. As such, with adequate precautions (e.g., trace-metal clean procedures), data on multiple elements can be generated automatically when measuring elements of traditional interest such as P. The extent to which ionomic data, capturing dynamics in elements other that the commonly measured or manipulated ones, explains patterns at higher levels of organization (i.e., community, ecosystem) remains to be seen. The prevalence of co-limitation in such studies (e.g., Harpole et al. 2011) suggests that an ionomic approach would be rewarding.

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Crichton RR (2017) Metal toxicity - an introduction. In Metal Chelation in Medicine (Eds: Crichton R, Ward R) Pp, 1-23. Royal Society of Chemistry, London.

Droop MR (1973) Some thoughts on nutrient limitation in algae. J Phycol 9, 264–272.

Elser JJ, et al. (1996) Organism size, life history, and N:P stoichiometry: towards a unified view of cellular and ecosystem processes. Bioscience 46, 674–684.

Harpole WS, et al. (2011) Nutrient co-limitation of primary producer communities. Ecol. Lett., 14, 852-862.

Hessen DO, Anderson TR (2008)) Excess carbon in aquatic organisms and ecosystems: Physiological, ecological, and evolutionary implications. Limnol. Oceanogr. 53, 1685-1696.

Jeyasingh PD, et al. (2011).). How do consumers deal with stoichiometric constraints? Lessons from functional genomics using Daphnia pulex. Mol Ecol 20: 2341-2352.

Salt D, et al. (2008) Ionomics and the study of the plant ionome. Annu. Rev. Plant Biol. 59, 709–733.

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Selected Recent Stoichiometry Publications

Bumpers, P.M., A.D. Rosemond, J.C. Maerz, and J.P. Benstead. 2017. Experimental nutrient enrichment of forest streams increases energy flow to predators along greener food-web pathways. Freshw. Biol. 62: 1794–1805. doi:10.1111/fwb.12992

Butler, P.M., T. Lewis, and C. Chen. 2017. Fire alters soil labile stoichiometry and litter nutrients in Australian eucalypt forests. Int. J. Wildl. Fire 26: 783–788. doi.org/10.1071/WF17072

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Di Palo, F., and D. A. Fornara. 2017. Plant and soil nutrient stoichiometry along primary ecological successions: Is there any link? PLoS One 12: e0182569. doi:10.1371/journal.pone.0182569

Erga, S.R., S.B. Haugen, G. Bratbak, J.K. Egge, M. Heldal, K.A. Mork, and S. Norland. 2017. Seasonal variations in C:N:Si:Ca:P:Mg:S:K:Fe relationships of seston from Norwegian coastal water: Impact of extreme offshore forcing during winter-spring 2010. Mar. Chem. In Press: 1–11. doi:10.1016/j.marchem.2017.07.001

Fernandes, T.V., M. Suárez-Muñoz, L.M. Trebuch, P.J. Verbraak, and D.B. Van de Waal. 2017. Toward an ecologically optimized N:P recovery from wastewater by microalgae. Front. Microbiol. 8: 1742. doi:10.3389/fmicb.2017.01742

Filipiak, M., K. Kuszewska, M. Asselman, B. Denisow, E. Stawiarz, M. Woyciechowski, and J. Weiner. 2017. Ecological stoichiometry of the honeybee: Pollen diversity and adequate species composition are needed to mitigate limitations imposed on the growth and development of bees by pollen quality. PLoS One 12: e0183236 doi:10.1371/journal.pone.0183236

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Phillips, K.N., C.M. Godwin, and J.B. Cotner. 2017. The effects of nutrient imbalances and temperature on the biomass stoichiometry of freshwater bacteria. Front. Microbiol. 8: 1692. doi:10.3389/fmicb.2017.01692

Schmitz, O.J., and A.E. Rosenblatt. 2017. The temperature dependence of predation stress and prey nutritional stoichiometry. Front. Ecol. Evol. 5: 73. doi:10.3389/fevo.2017.00073

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